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EFFECT OF URBAN OZONE LEVELS ON LABORATORY-INDUCED RESPIRATORY INFECTIONS

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SUMMARY

The effect of the time of exposure to an aerosol of viable microorganisms on the incidence of respiratory infections associated with a 3 h exposure to ozone (O_3) was studied. The 157 and 196 $\mu g/m^3$ (0.08-0.1 ppm) levels of O_3 used occur regularly in some urban communities. The studies reported here show that the susceptibility of mice to a laboratory-induced infection can be maximally enhanced if the infectious challenge is concurrent with the exposure to O_3 .

INTRODUCTION

The complex relationships between the concentration of an environmental chemical, the length of exposure, and the pattern of the exposure regimen have been shown to play a major role in determining the nature of the response to the toxicant [1,10,19]. A sensitive method for evaluating the inhalation effects of gaseous and particulate pollutants involves determining the potential of chemical agents for altering host susceptibility to respiratory infectious agents. Traditionally, in this microbial-host interaction system, the laboratory induced infection is administered immediately upon the cessation of the pollutant exposure [3,6,15]. But as dose-response studies approach the no measurable effect level, it becomes increasingly important to define better the components of the bioassay system which may be varied to provide increased sensitivity. The sensitivity of the model has been examined as a function of such factors as cold [2], exercise [13], and interaction with other environmental pollutants [7,12]. The studies reported here examine the rela-

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tionship of the time of the infectious challenge to a 3 h exposure to urban community levels of O_3 .

MATERIALS AND METHODS

For each replication of the experimental design, pathogen-free female mice (CD-1, COBS[®], Charles River Breeding Laboratory, Wilmington, MA) weighing 20–25 g (approx. 4–5 weeks old) were randomly assigned to various treatment groups. The exposure chambers used in these studies [17], as well as the method for generating O_3 [4], have been described previously. For calibration and monitoring of the chamber O_3 concentration, the recommended Federal Register procedure employing chemiluminescence was used [8]. A complete record of the chamber concentration profile was obtained using a strip chart recorder (Hewlett Packard, Model 680, Pasadena, Calif.) which was attached to the O_3 analyzer (Bendix, Model 8003, Roncerverte, W. Va.). The O_3 analyzer was calibrated weekly. In addition, the chamber O_3 concentration was periodically checked using the Saltzman method [18].

To insure uniform exposure to the pollutant, as well as to the bacterial aerosol, each of the 20 mice in an O_3 -exposed group was individually housed in an open-mesh stainless steel cage. An equal number of control mice were caged in the same manner but received filtered room air. In the first exposure regimen, the infectious challenge was administered at various time periods after the cessation of the 3 h exposure to $196 \mu g O_3/m^3$ (0.1 ppm). The control and O_3 -exposed mice were combined and challenged for 20 min to a viable aerosol of *Streptococcus pyogenes* (Group C) containing 10^9 cfu/ml. The animals were then separated into their respective treatment groups, and the incidence of mortality was observed over a 15-day period.

In another exposure regimen, after the first 30 min of the O_3 exposure, the 20 min infectious challenge was initiated and proceeded simultaneously with the gaseous exposure. The total length of exposure to O_3 was 3 h, and the O_3 concentrations studied were $157 \mu g/m^3$ (0.08 ppm) and $196 \mu g/m^3$ (0.1 ppm). Mortality rates were determined using 20 mice/group for the $196 \mu g O_3/m^3$ (0.1 ppm) exposures and 40 mice/group for the $157 \mu g O_3/m^3$ (0.08 ppm) exposures. With this regimen, in contrast to the above studies, the infectious challenge for the control mice could not be administered simultaneously with the O_3 -treated animals. In order to determine the influence of the initial number of bacteria deposited on the level of mortality, the number of cfu/lung was determined. The deposition of inhaled bacteria was measured using standard microbiological techniques [11]. The viable bacterial count was analyzed in triplicate for each mouse and expressed as the number of cfu/lung.

Since there was variability between replications of the experiment in the number and the virulence of the viable microorganisms aerosolized, a reduction in experimental error was achieved by expressing the data for each replication as the difference in mortality between the O_3 -exposed and control mice. The objective of the study was to establish the optimum time to ad-

minister the infectious challenge for a significant increase in mortality to be observed. A *t*-test was used to test the null hypothesis of no increase in mortality due to O_3 exposure for each of the time periods. The bacterial deposition data was analyzed with an analysis of variance which accounted for the assay error of the microbiological technique.

RESULTS

Fig. 1 shows the relationship between the time of the infectious challenge to a 3 h exposure to $196 \mu g O_3/m^3$ (0.1 ppm) and the resultant mortality. When the infectious challenge was given either immediately, 2, 4, or 6 h after cessation of the pollutant exposure, the average difference in mortality varied from a low of $2.0\% \pm 5.7$ SE to a high of $6.7\% \pm 3.6$ SE. Of these bacterial challenge times, only the 2 h post-exposure period produced a statistically significant ($P < 0.05$) increase in mortality (6.7%). However, if the mice were given the infectious challenge concurrently with the 3 h exposure to $196 \mu g O_3/m^3$ (0.1 ppm), a highly significant ($P < 0.01$) increase in mortality of $21.0\% \pm 5.3$ SE was observed.

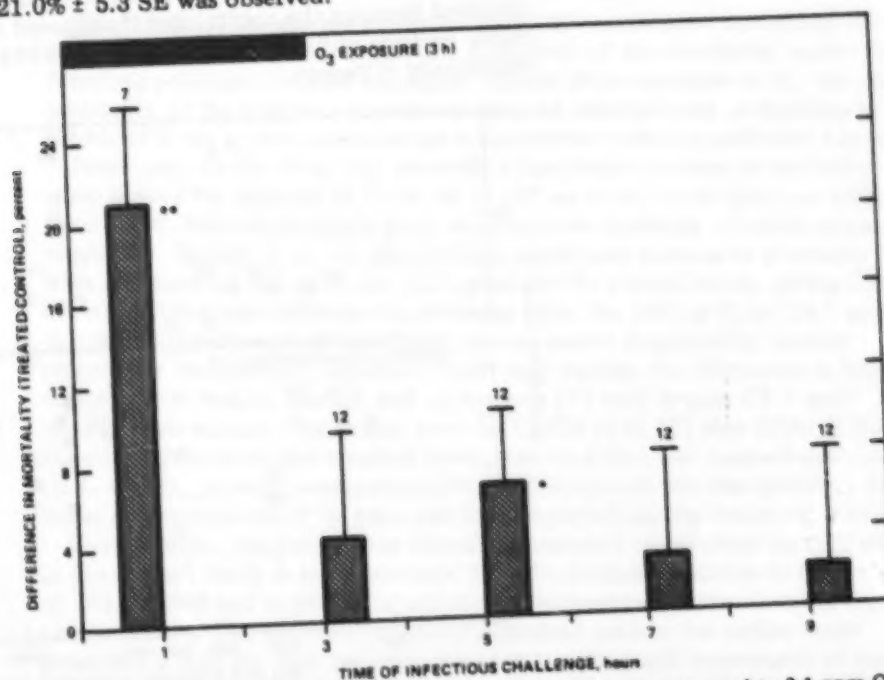


Fig. 1. Difference in mortality (O_3 -exposed minus control) in mice exposed to 0.1 ppm O_3 as a function of the time of infectious challenge. Numbers atop standard error bars indicate the number of replicate experiments where each replication used 20 mice/group. *Significantly greater than zero, $P < 0.05$. **Significantly greater than zero, $P < 0.01$.

This latter effect cannot be attributed to the difference between the control and O_3 -exposed mice in the number of viable microorganisms initially deposited. Immediately after the cessation of the bacterial challenge, the number of viable microorganisms per lung was $1249 \text{ cfu} \pm 104 \text{ SE}$ for the control mice and $1656 \text{ cfu} \pm 86 \text{ SE}$ for the $196 \mu\text{g } O_3/\text{m}^3$ (0.1 ppm) exposed mice. Cumulative data from control mice illustrate that within the range of 200–4000 cfu/lung, there is no relationship ($r = 0.002$) between the number of cfu/lung and the resulting level of mortality (Fig. 2). The overall mortality based upon the 2650 control mice represented in Fig. 2 averaged 27.7%. These scattergram data clearly show that the number of cfu measured in these experiments (1249 cfu for control mice vs. 1656 cfu for O_3 -exposed mice) do not influence the mortality rate. Hence, the significant increase in the observed mortality must reflect the effect of the O_3 exposure.

Using the experimental regimen wherein the infectious challenge was administered concurrently with the pollutant exposure, studies were also conducted at $157 \mu\text{g } O_3/\text{m}^3$ (0.08 ppm). As evidenced by the data in Table I, with 12 replications of the experimental design there was a statistically significant ($P < 0.05$) increase in mortality over control of 5.4%. The difference observed between the control and O_3 -exposed mice in the average number of viable bacteria initially deposited per lung (879 cfu vs. 926 cfu) was not statistically different.

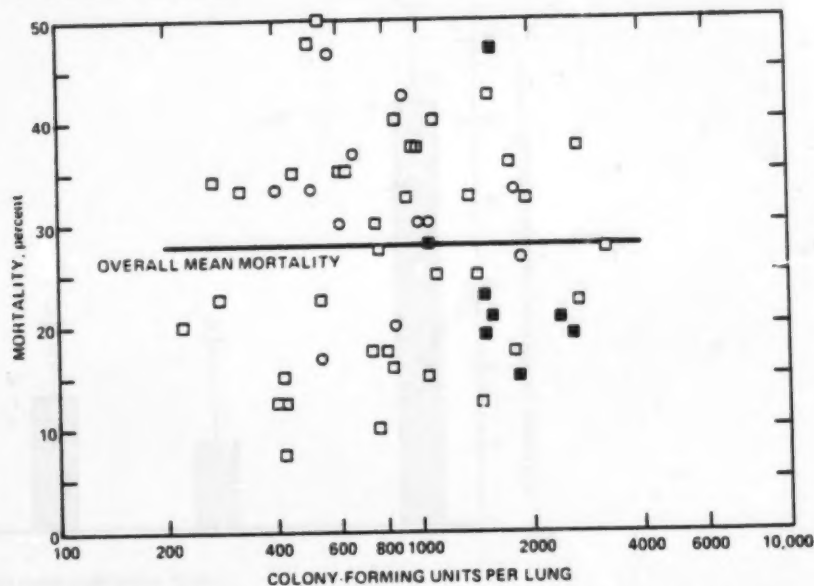


Fig. 2. Relationship between the number of viable microorganisms deposited initially in the lung and the resulting mortality. The correlation coefficient of 0.002 shows no relationship between these variables. ■, □, and ○ represent the data from experiments which used 100, 40, and 30 control mice, respectively.

TABLE I

SUMMARY STATISTICS FROM INFECTIVITY STUDIES ON MICE EXPOSED TO 0.08 ppm O₃^a

Treatment group	Number of viable microorganisms per lung initially deposited (cfu/lung)	Mortality (%)	Difference in mortality (O ₃ -Control) (%)
Control	879 ± 54	19.2 ± 1.8	5.4 ^b ± 3.0
O ₃ (0.08 ppm)	926 ± 82	24.6 ± 2.2	

^aData are mean values ± SE. Control and O₃ treatment bacterial deposition means are based upon 25 and 19 mice, respectively. Mortality means are from 12 replicate experiments with 40 mice/group.

^bMean difference in mortality is significantly greater than zero ($P < 0.05$).

DISCUSSION

The results of the studies presented here have established one of the factors which may be varied to alter the sensitivity of the infectivity model for detecting pollutant induced biological effects. With exposure to O₃, the susceptibility of the host to a laboratory-induced infection can be maximally enhanced if the infectious challenge is concurrent with the pollutant exposure.

Previously, Coffin et al. [5] observed a significant increase in mortality when mice were exposed to O₃ levels of 157 µg O₃/m³ (0.08 ppm) or higher for 3 h and then immediately given an infectious challenge of viable microorganisms. Ehrlich et al. [7] also found a significant increase in mortality with exposure to 196 µg O₃/m³ (0.1 ppm). In the current study, giving CD-1 mice the infectious challenge immediately after the 196 µg O₃/m³ (0.1 ppm) exposure did not result in significant enhancement in mortality. Several reasons are immediately apparent which may explain the difference in these experimental results. Ehrlich and co-workers [7] used female CF-1 and CD₂F₁ mice in their studies. The earlier work by Coffin et al. [5] also differed from the current study in that a mouse strain was used that was nonpathogen free (D.L. Coffin, personal communication). Differences in the susceptibility to lethal concentrations of O₃ have also been reported among strains of mice [14].

Furthermore, the continuous chemiluminescence monitoring method used in the current study is more accurate than the method available to Coffin et al. [5] in 1968 and is able to detect minor fluctuations in the chamber O₃ concentration. The periodic monitoring method used in the earlier study possessed a long lag time between chamber sampling and adjustment of the chamber concentration. Thus, the possibility exists that increased chamber O₃ levels may have been present in the Coffin et al. study. Concentration spikes were not present in the current study, as evidenced from the chemiluminescence monitoring data. For the 157 µg O₃/m³ (0.08 ppm) exposures, the chamber O₃ concentration ranged from 145 to 165 µg/m³ (0.074–0.084 ppm). Based upon

15 min sampling averages, the chamber O_3 concentration averaged $157 \mu\text{g}/\text{m}^3 \pm 2 \text{ SE}$. Similar monitoring data for the $196 \mu\text{g } O_3/\text{m}^3$ (0.1 ppm) exposures had a standard error of $6 \mu\text{g}/\text{m}^3$.

These differences aside, it should be re-emphasized that by modifying the experimental technique (the infectious challenge was given concurrently with the O_3 exposure) we have also demonstrated that a statistically significant enhancement in mortality results in mice exposed to 157 or $196 \mu\text{g } O_3/\text{m}^3$ (0.08 or 0.1 ppm) for 3 h. The infectivity model can be used to assess pollutant effects even when significant changes are made in the technique. The model is supported by experimental evidence which shows that pollutants which cause an enhancement of mortality in the infectivity system also cause reductions in essential host defense systems [9,11,16], and thus, the model is a sensitive bioassay system available to environmental toxicologists.

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